S8.P3

Succinate:menaquinone oxidoreductase in *Bacillus subtilis* membrane vesicles is activated by ATPase-generated $\Delta\mu H^+$ with no effect on the fumarate reductase activity

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Succinate:quinone oxidoreductases (SQR) from Bacilli catalyze energetically unfavorable reduction of menaquinone by succinate and this activity is lost upon $\Delta\mu H^+$ dissipation, for example during isolation of leaky membranes from the cells [1]. As shown in [2], tightly coupled membrane vesicles from *Bacillus subtilis* retain the ability to respire on succinate with the rate approaching that of the whole cells. Here we report that in the tightly coupled vesicles from *B. subtilis* SQR can be activated by membrane energization by virtue of ATP hydrolysis.

Respiratory chain of *B. subtilis* strain with cytochrome *bd* as the only terminal oxidase generates $\Delta\mu H^+$ with low efficiency ($1H^+/e^-$) and, contrary to the wild type, mutant membranes reveal no succinate oxidase activity even under tightly coupled conditions. Addition of ATP to the inside-out vesicles results, first, in membrane energization and, second, in the ability to oxidize succinate. Subsequent uncoupling inhibits succinate respiration completely. On the other hand, neither ATP-driven energization nor uncoupling affects the reversed, fumarate reductase, activity of the membranes. The latter fact is in accordance with our finding that the menaquinol: fumarate reductase activity of *B. subtilis* membranes is not coupled to $\Delta\mu H^+$ generation.

In order to identify the potential $\Delta\mu H^+$-sensitive step(s) of the succinate:menaquinone reaction, the redox state of the di-heme cytochrome *b* forming the membrane anchor part of SQR was assayed. To this end, membranes were equilibrated with the succinate/fumarate redox couple so that the reduction of the high (b_H) and low (b_L) potential hemes were near 100% and 50%, respectively. The membranes were then energized by addition of ATP. The energization did not result in any change in the reduction level of the hemes, as well as subsequent uncoupling. Presumably, the effect of the ATPase-imposed $\Delta\psi$ on electron distribution among the redox centers of SQR is electrically compensated by intraprotein movement of positive charges (probably, protons), which could be a constituent part of the catalytic mechanism.

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S8.P4

The photosynthetic electron transfer chain of *Heliobacterium modesticaldum*

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Heliobacteria are anoxgenic photosynthetic bacteria that perform cyclic electron transfer around their photosynthetic reaction center. The reaction center is a homodimeric type I RC transferring electrons from the primary donor chlorophyll to the iron sulfur center F_x and further on to one of two soluble 8Fe8S ferredoxins [1]. The primary electron donor gets re-reduced by a membrane attached mobile mono-heme cytochrome *c*, itself reduced by a Rieske/cyt*b* complex with a dimeric cytochrome *c* subunit, a split membrane part harboring two *b*-hemes and heme c_i [2]. To interpret flash induced kinetics, knowledge of the redox midpoint potentials of the cofactors of the reaction chain is useful. We therefore proceeded to optical and EPR redox titrations. Whereas the potentials found for the Rieske protein (+200mv vs SHE) and the *c*-hemes were close to the values previously reported for *Heliobacillus mobilis* the potentials of the *b*-hemes were evaluated to −350 and −210 mV, much lower than so far suggested We will discuss the potentials and kinetics obtained on *Heliobacterium modesticaldum* reaction center and Rieske/cyt*b* complex under different growth conditions with a particular attention to the role of heme c_i and the pathway electrons taken from ferredoxin to the menaquinone pool.

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S8.P5

Urocanate as a novel electron acceptor for anaerobic respiratory chain of *Shewanella oneidensis* MR-1

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We performed heterologous expression of the gene of the hypothetical UrdA (SO_4620) protein from *Shewanella oneidensis* MR-1 [1]. The recombinant protein was purified and investigated. It was shown that UrdA contains two flavin prosthetic groups: a noncovalently bound FAD and a covalently bound FMN residue. Redox titration of these centers at pH 7.5 showed that the noncovalently bound FAD operates as a two-electron carrier with midpoint potential of −280 mV, while the covalently bound FMN residue is capable of two sequential one-electron reduction steps (Em1 = −225 mV and Em2 = −250 mV) with transient formation of the anionic semiquinone. Using structural modeling and comparative genomic analysis we proposed that UrdA catalyzes reduction of urocanate in bacterial cells. The following experiments confirmed this prediction and showed that UrdA does catalyze the unidirectional reaction of 2-electron reduction of urocanic acid to deamino-histidine, an activity not reported earlier. UrdA exhibits both high substrate affinity and high turnover rate ($Km << 10\,\mu M$, $kcat = 360\,s^{-1}$) and strong specificity in favor of urocanic acid. UrdA homologues are present in various bacterial genera, such as Shewanella, Fusobacterium, and Clostridium, the latter includes the human pathogen *Clostridium tetani*. The UrdA activity in *S. oneidensis* is induced by its substrate